rRNA Gene Cluster

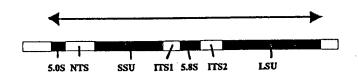


FIG. 1

_	73'	CAA:	3'	
1		1		50
AAAGTCGCAC	CTTTCCCCAT	AAACCCCCTC	ACCCCCT	TGGACATTGT
51		`) <u> </u>		100
TCCACTTTTC	ACTTGTATTG	TGAAGCACCC	AATGCTAGCC	
101				150
TCCAGTAGTT	CAATAGAGAG	ACTAGTGAAC	ATAGTTTATA	ACATTGTCCA
151				200
AGGGGTGGAG	GGGGATGCGC	GAAATCGATG	TGCACGTTTG	GTCAAAGATG
201				250
CTCGCGAAAG	CTGCACATCA	ATTTCGCACA	TGGGCGAAAT	TGACTTGCAG
251				300
	AAGTTGATGT	AGGCCATGTG	GCTCGATTC	AACCATATGG
301				350
GTATGCTTCT	GAGGATGGGG	TGTTACAGTG	GACCATATGA	GGTAGGTCAT
351	~1.~~1.1.m	GMGM3.3.MGM	00003 888003	400
TTGGAGATGT	CACCAAAATG	GTCTAAATCT	GCGCATTCCA	TTTAAGTGAA
401			111mmo100m	450
	ATTTAAGTGA	ATTTTACTTA	AAATTGACCT	TTTTCGTTGC
451	aamaamaa ma	000000000	OC 3 3 MMMMMM	500
GCAGATTTGG	GGTGGTGATG	GGTGACGCGG	CGAATTTTT	AAAAAAGAGG 550
500	coma mmmoma	ттттт т	CA CCCCCCCCA	
TATATCGCGT	GCTATTTGTA	TTTTTGGTAT	CACCGCGTCA	CCAATCACCA 600
551		GTTTTTCCGG	3 mm 3 mm < < 3 m	TTTTTTTTTT
TTGACGGTTT 600	CTTTTTCGAA	GITTITCCGG	ATTATTGCAT	650
	GCTGATTCTT	CCCNANCCAC	TGTTGTGATG	TCCGAGTTCC
ATTGTGGGTG 651	GCTGATTCTT	GCGAAAGGAC	IGIIGIGAIG	700
CAAATTGGGA	GTTTTTGGAC	ATCACTCCTG	метессее	GGCGATCAGG
700	GIIIIIGGAC	ATCACTCCTG	ATCTGCCGGC	750
ATGACTGACA	TTTCGATATA	TTTTGGGTAT	TCGATAGCTG	CCAAATCGGT
751	IIICGAIAIA	IIIIGGGIAI	ICGAIAGCIG	800
CAGCGTCGAG	TATTCCGGTT	TATTCGAAGG	ATTCATGATA	TTGCAAAATA
800	IMITCCOOTI	1111110111100		850
TCATTGATTT	TCATGGGGTT	TTGTATTAGT	ACCCGCTCAT	TGTGGGAAAG
851	1011000011		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	900
TCGGGTGGAT	TTATCTTACC	CGCAAATCTA	ATACAAGATT	TGCATGATGC
900		***************************************		. 950
AGCAATAGAC	CAAGGTTAGT	ATAGCAGTTG	TATTTATACG	ACTAGTTATG
951	3121331			1000
CAAACCCTTT	GTGTTTTTTG	TTGCGACTCT	TGGCGTGAAC	CGGAAGACCG
1000				1050
GACCTCGCTT	TCGACTATTC	ATCTTTGATG	GATATGAGAT	CGCAAGGGTA
1051				1100
TCGCTTCGTG	CGATATTTAG	TGACCATCAG	AGCACGCTAC	GACTTTTGAT
1100				1150
TATATCCTTG	GATTTAATCG	GAAGCTCGCA	AGCATTGCAT	TGATGCAATC

FIG. 2

CATTIT TGCTTTCACA ACCCCGCACC CCATGTACAA TGTTGCCAAC #1 CACTAGAGTT TCAACAACAT TCGGATTTGA CAACATGTCA ACAATTCACA #51 ACAGAAATTG ACAACATTGT CACAAATTCT CAAATTGGAC AACATTGGAC AAAAATTCAC AACATACATT GGACAACAGT GGACAACGAA CCCAAACCCG #151 ACAACATTGT CCAGGGGGAT AGGGGGTGAA AAAGCAGTGC CGGCAAAGTC GAAAGATGTC AAGTTGGAAT GCGGCTCAAA TTCGTCATTT GTGTAAATCC #251 GCAATTTTGC CAATGTGCAA TTTTGCAAAT GTGCAATTTT GCAAATGTGC #301 AATTTTGCCA ATGTGCAATT TTGCAAATGC GCAATTTTGC AAATCCGCAA #351 TTTTGCAAAT GTGCAATTTT GGAAAATCAC CAAATGAAAA TCGTCCAAGT #401 CGAATTGGAG GCGTGGTGAC ATGGTCCCGG GATCCCCTGG TTACAGTGGA CAATATCCCA GCAATATTCG CTGTAATTTG GAGTTTCGCT GTTTTGGCAA #501 ATTTTGAGTC TGAAAAAAAA AATTGCAAAT GCGCAAAGGG GGTGAAGGAA #551 AAAAAAGCAC CCCCGAAGGT AAAATTCCCT TTAAGTCCCT TGCGCATTTG #601 CAAAATTTTC AAAAATTGTT GCAAATGCGC TTTTGTTATT TGGCCGGTTC #651 ATTGGTGTCA AAAGTTGCCT GGGGTGGTTA CACAATGCAC GGAATTGGTT : GGAAGTTGTG TGATTGAAAA TTGGTCGTGT CACACAATTT TGCGCATTTG #751 CAAAAATTCG CAAATTGGAC AAAAAAGGGT CGCGCACAGT CAAATTGCGC #801 AAATTTCACT TTGAAGTGAG TGCGCATTTG TGGGGCAGAA ATGTGGTGAC #851 AGCATCGTTT TTTATAATAA ATATTCTATA TTTAGTATCT TTATTATAAT #901 TTGCTGTCAC CAATCACCAT TTTAGAATTT TTATTTTTTT ATGTTTTAGT #951 GACCGCGGGA TTTTTTGCAA AGTACTATYG TGATGTTTGA GTTGTTTGAA #1001 ATGGGCAATT TAGAACATCA TCAGAAATCG CTGAATAGTG ATTTTTGAGT #1051 TTGACTGTTT GAAGTGTTTT GGGTATTCGG CAGCTGCCAA ATCGGTCAGC #1101 GTCGAATATA ATAGCATTTT TGTGTGTATA TGATATTTAG CGATATCATT #1151 GGAATCATGG GGTTTTGTAT TAGTACCCGC TCATTGTGGG AATGTCGGGT #1201 GGTTCAATAT CACCTGCAAA TTTAATACAG GATTTGCATG ATGCAGCGAC #1251 TGACCGGGGT TGGTATAATA GCTGATTATT CGGCTTATTA TGCAGACCTA #1301 TCGTGTTAGT AGTTGCGACT CTTGGCGTGA ACCGGAAGAC CGGAACTTGA ATTCGACTAT TTACGTCCGT AAACAGGAGA TTTCAAGAAT ATTGCACATT : #1401 TTGCGTGATA TAAACGTGAT CATCTGAGCA CGCTTCGACT CTTGGATATC #1451 TGCTAATCAG CCGTCATCTG AGAGCTCGCA AGCATTGCAA TTGATGCAAT #1501

1				50
CGTGCCCTTT	TCACGAATTC	ACAGCCCCGC	CCCATGTA	CAATGTTGCC
51		•		100
CACCCGAAAT	GCCTGCCTGC	CCACCCGAAA	TGCCCGAAAT	GCCCGTTAGA
101				150
AAAAGTATGC	GAAAAGTTCT	TGTCAATTTT	GACAGTGTGT	GAAAAAACTG
151				200
AAAAAGTCCA	CTCAACATTG	CATTATGCAA	TTTGCCACTC	AACATTGTCC
201			*	250
AGGGGGATAG	GGGGTGAAAA	AGTATCGCAG	TCCAACTGAA	AAGATGCTAA
251				300
GTTGAAATGC	GGCGCAAATT	CATCACTTGA	GTTGCGAAAA	TCCCTAAAGT
301	00000111111			350
CGAATTTGGC	ACTCGGTGAC	ATGATCGGGA	ATTTCCCTCC	TTACAGTGGT
351	ACTCGGTGAC	111011111111111111111111111111111111111	11111000100	400
	CAATTTTGGC	AAAGTTTTTG	A CHIMING COAC	TTTTCGCAAA
	CAATTTIGGC	AAAGIIIIIG	AGITICGCAC	
401	~~~~~	mmmc	0000111000	450
	GAAAAAAAA	TTTCAACTTT	GCGCAAAGGG	GTCAAAGGGA
451				500
AAAAAAGCAC	CCTCAAAAGG	AAATTTCCCT	TTAATCCCCT	TTGAAAAAA
500				550
TGCGCAAAGT	TAAATTTGCG	AAAATTTCGA	TTTTCTCATA	TGACCGATTA
551				600
GTTGGTGCCA	GATGGTAGTC	GGGATGGTTA	CACGGTGCAC	GGAACTCGTT
600				650
GGAAGTTCTG	GAGTTACGAA	TTGGTCCCGT	CACCACAATT	TGCGCATTTT
651				700
TGAAATTGCG	CAAATTTGCG	AAAAAAGCAG	CGCGCAAAGT	TAAATTGTGC
700				750
GAAAATTGAC	TTTCAGGTCG	GTGCGCAAAT	TTGGGGTGAA	AAAGTGGTGA
751				800
	ATTATAATAA	ATAATCTATA	ATCTAGTTCT	TTTATTATAA
800				850
	CCAATCACCA	ጥጥፕGAGAጥጥጥ	TTTATTTTTT	TATGTTTTAG
851	CCIMITCHICCH			900
TGACCGCGGT	ል ተተመተመተመር C A	GAGTACTATC	GTGATGTCTG	AGTTGTCTAA
900	ATTITIOCH	01101110111110	01011101010	950
AACGGCAATT	TCAGAACATT	ACCAGAAAAC	ልርጥርል አጥልርጥ	GGTTTCTGAG
951	ICAGAACAII	ACCAGAAAAC	ACIGAMIAGI	1000
TCTGACTGTT	mc a a cmcmmm	TGGGTATTCG	CCACCTCCCA	
1000	IGAAGIGIII	IGGGIATICG	GCAGCIGCCA	1050
	ACTAACATTT	CTGTGTGTAT	ATGGTATTTA	
	ACTAACATTT	CIGIGIGIAI	AIGGIAIIIA	1100
1051	CCCMMMMCM3	mm3.cm3.cccc		
	GGGTTTTGTA	TTAGTACCCG	CICATIGIGG	
1100				1150
	TCACCTGCAA	ATTTAATACA	GGATTTGCAT	
1151	·			1200
	TTAGTATAAT	AGCTGATTAT	TCGGCTTATT	
1200				1250
ATCGTGTTAG	TAGTTGCGAC	TCTTGGCGTG	AACCGGAAGA	
1251				1300
ATTTCGACTA	TTTACGTCCG	TAACACGTCC	GTAAACAGGA	GATTTCAAGA
1300				1350
ATATTGCACA	TTTTGTGTGA	TATAATCGTG	ATCATCTGAG	CACGCTTCGA
1351				1400
CTCTTGAATA	TTTGTTAAAC	AACCGATATT	CGGGAGCTCG	CAAGCATTGC
1400				1450
AATTGATGCA	ATC			

FIG. 4

Primer	Sequence	Target
300 F	5'-CACTTGTATTGTGAAGCACCC-3'	
300 R	5'-TTG GTG ACA TCT CCA AAT GAC-3'	Perkinsus marinus
500 F	5'-ATGCTAGCCCATAGAACAGT-3'	, ommode mamae
500 R	5'-ATGCTAGCCCACATCACAGC-3'	
NTS7	5'-AAGTCGAATTGGAGGCGTGGTGAC-3'	
NTS6	5'-ATTGTGTAACCACCCCAGGC-3'	Perkinsus andrewsi
PM5	5'-ATGCTAGCCC ATAGAACAGT-3'	P. marinus type l
PM7	5'-CAT CTC CAA ATG ACC TAC CT-3'	P. marinus type I
PM6	5'-ATGCTAGCCC ACATCACAGC-3'	P. marinus type II
PM8	5"-CAT CTC CAA ATG ACC TAC CA-3"	P. marinus type II

FIG. 5

FIG. 6

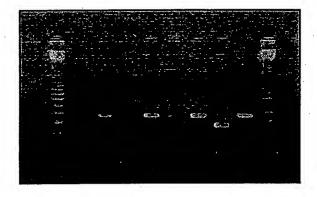


FIG. 7

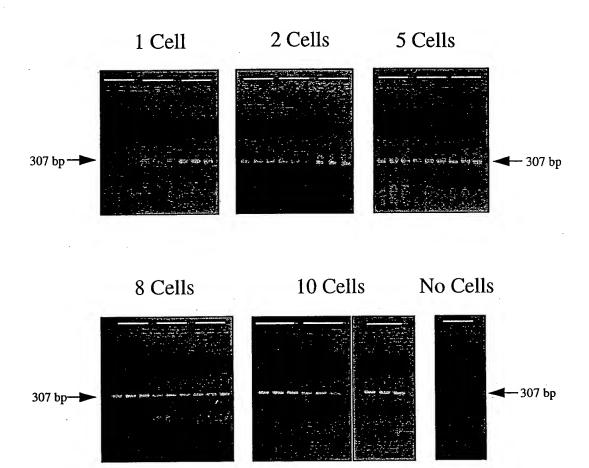
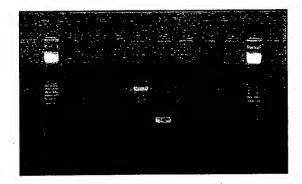
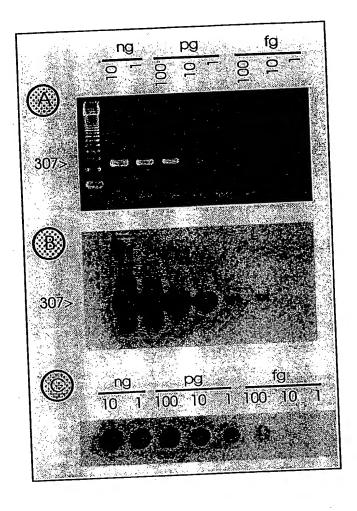


FIG. 8

Samples





	1		d		50
Type-I	CACTTGTATT	GTGAAGCACC	CAATGCTAGC	CCA T A GA ACA	GTCCAGTAGT
Type-II	CACTTGTATT	GTGAAGCACC	CAATGCTAGC	CCACATCACA	GCCAGTAGT
	51				100
Type-I	TCAATAGAGA	GACTAGTGAA	CATAGTTTAT	AACATTGTCC	AAGGGGTGGA
Type-II	TCAATAGAGA	GAC G AGTGAA	CATAGTTTAT	AACATTGTCC	AAGGGTGGA
	101				150
Type-I	GGGGGATGCG	CGAAATCGAT	GTGCACGTTT	GGTCAAAGAT	GCTCGCGAAA
Type-II	GGGGGATGCG	CGAAATCGAT	GTGCACGTTT	GGTCAAAGAT	GCTCGCGAAA
•	151			•	200
Type-I	GCTGCACATC	AATTTCGCAC	ATGGGCGAAA	TTGACTTGCA	GGTGGGTATA
Type-II	GCTGCACATC	AATTTCGCAC	ATGGGCGAAA	TTGACTTGCA	GGTGGGTATA
	201	4			250
Type-I	AAAGTTGATG	TAGGCCATGT	GGCTCGATTT	CAACCATATG	GGTATGCTTC
Type-II	AAAGTTGATG	TAGGCCATGT	GGCTCGATTT	CAACCATATG	GGTATGCTTC
	251				300
Type-I	TGAGGATGGG	GTGTTACAGT	GGACCATATG	AGGTAGGTCA	TTTGGAGATG
Type-II	TGAGGATGGG	GTGTTACAGT	GGACCATATG	TGGTAGGTCA	TTTGGAGATG
	301			***	
Type-I	TCACCAA				
Type-II	TCACCAA		•		

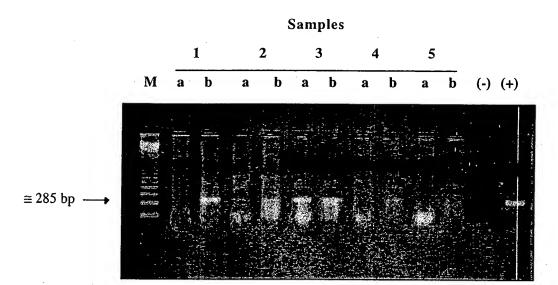
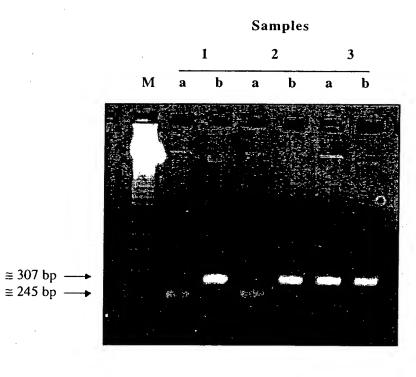


FIG. 12



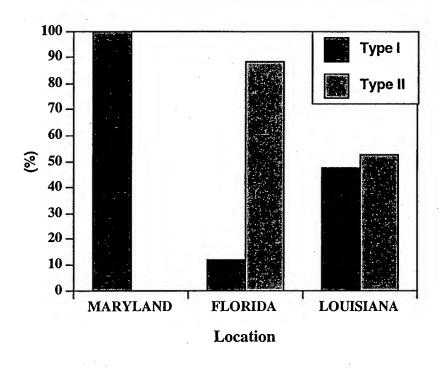


FIG. 14

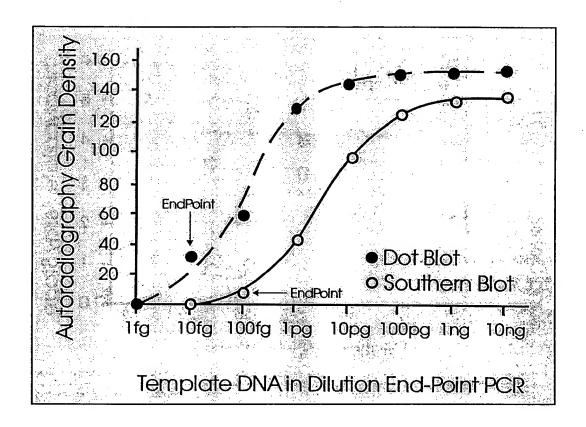
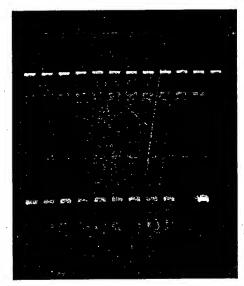


FIG. 15

Samples

1 2 3 4 5 6 7 8 9 10 11 12



13 14 15 16 17 18 19 20 1 - + -

FIG. 16



#1	.TCTTTTTAAA TCGCAC			
>P. atlanticu #51	18.CCCCTGGACA ATGTTA			
>P. atlanticu #101	18.AGTAGTCCAC TAGAGA			
>P. atlanticu #151	us.GGGAAAGGGG GGCGCG			
>P. atlanticu #201	s.AGTTTTGCTG CACCCC			
>P. atlanticu >PA690F-Text #251	s.GAGGGTAAAA GATGCTA ATGCTA	TGG TTGGTTGCGG	ACC	•
>P. atlanticu #301	s.CATCATTATC GAGGTCT			
>P. atlanticu #351	s.CGGAGGTGTC ACCACGG			
>P. atlanticu #401	s.TTTAAGTGAA ATTTAAG			
>P. atlanticu #451	s.GCAAAGTTGA GGTGGTG			
>P. atlanticu: #501	TATTTAT AAAAAATTAT.			
P. atlanticus #551	GGGCGTAATT TTCCGGG			
P. atlanticus #601	GGGGTAAATA GTGTCCG			
#651	GAGTTCCCAA ATTGAGGG		• • • • • • • • • • • • • • • • • • • •	• • • • • • • • •
#701	GTTTCAGATT TCCGACTT	• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •
#751	.AATCGGTCAG CGTCGAAT		• • • • • • • • • • • • • • • • • • • •	• • • • • • • • •
P. atlanticus PER1-Text #801	.GAGATATCAT TGGATTTC		TAGTACCC G	CTCATTGTG
P. atlanticus PER1-Text #851	.GGAAAGTCGG GTGAATTT G			
P. atlanticus PA690R-Text #901	.TGATGCAGCG ACTGACCG	GG GTGAGTGTAG CA	AGCTGTTCT AC	GGCTTGCT
P. atlanticus PA690R-Text #951	ACGCAGACCT ATCGTGTT.			
P. atlanticus. #1001	CCGGACCTCG CTTTCGAC	ra ttcattccga to	AATATGAG AT	TGCAAGGG
P. atlanticus. \$1051	TATCGCTTCG TGCGATAT	TT AGTGATCATC AG	AGCACGCT AC	GACTTCAG
P. atlanticus. PER2-Text	TATATCCTCG GATACACA	AGCTCGCAAG CA AGCTCGCAAG CA	TTGCATGA TG	CAATC

>P. #1	andrewsi-S		TCCTGCCAGT			GATTAAGCC
>P. #5			AGTATAAGCT			
>P. #1			AGTTTATTTG			
>P. #1			CTAATACATG			
>P. #2			CAGAACCAAC			
>P. #2!		. ATAATAACCC	GGCGAATCGC		CGGCGATGGA	
>P. #30			TCAGCTATGG			
>P. #3!			GGGAATTAGG			
>P. #40			TCTAAGGAAG			
>P. #45			TAGTGACAAG			
>P. #5(AGTAGATTTT			
>P. #55			CCAGCAGCCG			
	J3F-Text		GGTTAAAAAG	AGTTG	GATTTCTGCC	TTGGGCG
>P. #69			TCCTACGGGT			
>P. #70			TCGTGCTCAC			
>P.	andrewsi-S.	GACTTTTACT	TTGAGGAAAT	TAGAGTGTTT	CAAGCAGGCT	TATGCCGTG
#75	51					
>P. #80			ATGGAATAAT			
>P. #85			GAAGTAATGA			
>P. #90		TAACTGTCAG	AGGTGAAATT	CTTGGATTTG	TTAAAGACGA	ACTACTGCGA

FIG.18A

>P. andrewsi-S.AA #951		GTT TTCATTGATC		GTTAGGGGAT
>P. andrewsi-S.CG #1001	AAGACGAT CAGATAC			
>P. andrewsi-S.GG #1051	GATTGGGA GTCGTTA			
>P. andrewsi-S.AA #1101	AGTCTTTG GGTTCCG			
>P. andrewsi-S.GA #1151	ATTGACGG AAGGGCA			
<pre>>P. andrewsi-S.TC >SSU4F-Text #1201</pre>		ACC AGGTCCAGAC	ATAGGAAGG	
>P. andrewsi-S.GA #1251	TAGCTCTT TCTTGAT			
>P. andrewsi-S.GG #1301	TGGAGTGA TTTGTCT			
>P. andrewsi-S.GC #1351	TAAATAGT TGCGTGA			
>P. andrewsi-S.CT #1401	TTGTGTGT TTAACAC			
>P. andrewsi-S.CC #1451	CTTAGATG TTCTGGG			
>P. andrewsi-S.TA #1501	TTTCCTTG CCCGGTA			
>P. andrewsi-S.TA #1551	GGGATAGA CGATTGC			
		• • • • • • • • • • • • •		
>P. andrewsi-S.TT. #1701	AGTTCAGT TTCTGTT			CCTTATCACT
>P. andrewsi-S.TAG #1751	GAGGAAGG AGAAGTCO	GTA ACAAGGTTTC	CGTAGGTGAA (CCTGCAGAAG

>P. andrewsi-S.GATCATTC

FIG. 18B

ACACCGATTC ATTCTCTGAG AAACCAGCGG TCTCTGTAAA AGGAGATGGG #1

ATCTCCGCTT TGTTTAGATC CCCACACCTG ACCGCTTTAA CGGGCCGGGT #51

AGGTGCATAA CTTCTATGAA CCAATTGTAC TAGTCTAAAG TATCCAATAT #101

CCTTTTGGAT TTTGGTATTT CAAAACGAAA TTCCAAACTC TCAACGATGG #151

ATGCCTCGGC TCGAGAATCG ATGAAGGACG CAGCGAAGTG CGATAAGCAC #201

TGCGATTTGC AGAATTCCGT GAACCAGTAG AAATCTCAAC GCATACTGCA #251

CAAAGGGGAT TTATCCTCTT TGTACATACA TATCAGTGTC GCTCTTCTTC #301

CCGATACAAA CATTTTGTTG ATTTACAATC AACATTATGC TTTGTATCCC #351

GCTTGGATTC CTTTATTGGG ATCCGCTGTG TGCGCTTGCT GACACAGGCG #401

CATTAATTTG CAAGGCTATA ATACTACTGT ACTGTAGCCC CTTCGCAAGA #451

AGGACTGCGC TAGTGAGTAT CTTTGGATGC TCGCGAACTC GACTGTGTTG #501

TGGTTGATTC CGTGTTCCTC GATCACGCGA TTCATCGCTT CAACGCATTA #551

TGTCAAATTT GATGAATGCA GAGAGTTGTT TATGAATTAC GCGATCGCTT #601

TGGTCTCAGA ATCGTTACTA TAGCACGCTT GTCGGTTTGC AACCTGGCAA #651

TATGTCATCA TT #701

FIG. 19

						Primers to claim			
Perkinsus species	PCR	Name	Forward Primer (5'-3')	Position ¹	Name	Reverse Primer (5'-3')	Position ¹	Amplicon Size (bp)	Publication
Perkinsus marinus	Species	300F	CAC TTG TAT TGT	08-09	300R	TTG GTG ACA TCT	346-366	307	Marsh et al.
	specific		GAA GCA CCC			CCA AAT GAC			J. Parasitol. 1995 81(4):577-83.
									Robledo et al.
									J. Parasitol. 1999 85(4):650-6.
Perkinsus atlanticus	Species	PA690F	ATG CTA TGG TTG	262-283	PA690R	GTA GCA AGC CGT	933-952	169	Robledo et al.
	specific		GTT GCG GAC C			AGA ACA GC			J. Parasitol. 2000 86(5):972-8
Perkinsus andrewsi²	Species	NTS7	AAG TCG AAT TGG	447-470	NTS6	ATT GTG TAA CCA	717-736	290	Coss et al.
	specific		AGG CGT GGT GAC			CCC CAG CG			J. Euk. Microbiol. <u>2001</u> [(In Press)] <u>48:52-</u> <u>61</u>
Perkinsus marinus	Generic	PERI	TAG TAC CCG CTC	827-845	PER2	TGC AAT GCT TGC	1123-1139	313	[Coss et al.
			AT(TC) GTG G			GAGCT			J. Parasitol. (Submitted)]
Perkinsus atlanticus	Generic	PERI	TAG TAC CCG CTC	833-851	PER2	TGC AAT GCT TGC	1121-1137	305	[Coss et al.
			ATT GTG G			GAGCT			(Submitted)]
Perkinsus andrewsi	Generic	PERI	TAG TAC CCG CTC	1121-1239	PER2	TGC AAT GCT TGC	1523-1539	319	[Coss et al.
			ATT GTG G			GAGCT			J. Parasitol. (Submitted)]

Relative to the NTS sequence

²Perkinsus sp. (Macoma balthica)

FIG. 20

					Primers	Primers to claim		
Perkinsus species	PCR	Name	Forward Primer (5'-3')	Position	Name	Reverse Primer (5'-3')	Position ¹	Publication
Perkinsus andrewsi	Sequencing	SSU3F	AGT TGG ATT TCT GCC TTG CGC G	626-647	SSU4F	ACC AGG TCC AGA CAT AGG AAG G	1218-1239	Coss et al. J. Euk. Microbiol. 2001 [(In Press)] 48:52-

FIG. 21

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